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The goal of this research training program is to produce highly qualified scientists for careers as independent investigators in the field of breast cancer. In the last 20 years, there has been a steady increase in the incidence of breast cancer. During the same period, there has been a fundamental revolution in the understanding of molecular and cell biological concepts related to cell growth, function and tumorigenesis. To utilize what has been learned and to continue future progress in the area of breast cancer requires the continued availability of well-trained, innovative and committed scientists. This program represents an interdepartmental training program involving thirteen investigators from six departments. Trainees are predoctoral fellows with backgrounds in biochemistry, cell and molecular biology, molecular genetics and molecular virology. The training program provides trainees with additional foundation in carcinogenesis and breast cancer. In addition to the core curriculum taken by the predoctoral fellows in their respective academic departments, program enhancement is provided through trainees' participation in a graduate course on "Molecular Carcinogenesis," a Breast Cancer Seminar Series, and participation at national meetings, and local seminars. Six predoctoral trainees are currently enrolled in the training program.

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Daniel Medina July 17, 1997

PI - Signature Date

4. TABLE OF CONTENTS

5. INTRODUCTION:

Breast cancer is a complex disease whose ultimate understanding will require the integration of facts resulting from a multidisciplinary approach. Continued basic science research will provide a fuller understanding of the basic mechanisms of breast cancer which is necessary to conquer the disease in humans. In order to have the scientific human armamentarium to further this understanding, this training grant focuses on producing qualified scientists for careers as independent investigators in the area of breast cancer. The rationale for a targeted training grant in breast cancer is based on the belief that the elucidation of how oncogenes, tumor suppressor genes, hormones and growth factors act at the molecular level and as developmental-specific agents are critical questions directly relevant to the etiology, prevention, diagnosis, treatment and prognosis of human breast cancer. The training program draws together individuals who have an established research and training background in the mammary gland with individuals who have a research and training background in cell biology, molecular endocrinology, molecular biology, molecular virology, viral oncology, molecular genetics and biochemistry. The strength of the program is two-fold. First, the program brings together members of diverse disciplines to focus on the training of predoctoral students for careers in an area which, by its biological nature, is multi-disciplinary. Second, the program brings new intellectual approaches and insights to the problem of breast cancer which will be continued by the next generation of research scientists.

The design of the training program provides for trainees to be exposed to clinical problems and recent advances as well as the multi-disciplinary approaches to answering fundamental questions related to breast cancer research. The familiarity and close proximity of the training faculty facilitate and encourage the development of a new generation of research scientists who will be able to understand the problem of breast cancer at a more complex level and from a multi-disciplinary orientation.

6. BODY OF PROPOSAL

A. Trainees

The goal of this training program was to provide an environment for training in breast cancer research. To foster this goal, candidate graduate students had to meet a minimum set of requirements. Graduate students had to be at least in their second year of graduate school and had selected a thesis problem focusing on an aspect of mammary gland growth, differentiation and/or cancer. These students would be supported for two years by the training program provided they maintained satisfactory progress in their research program and they participated in the biweekly journal clubs and attended the course "Molecular Carcinogenesis". The progress and status of the first six students is summarized below. These students rotated off the training grant in July 1996. The summary of their research progress was provided in the last two progress reports, therefore the status only of these students is listed in Table 1. Three of the students have graduated or will graduate by June 1998 with the other three scheduled in 1999. Each student satisfactorily completed the requirements of the training program in the two years of support and maintained an active research involvement in breast cancer-related problems.

In July 1997, a second set of students rotated on the training program. Funding considerations resulted in one student, Jeff Jones, being maintained on the training grant for a second period of two years. The six students, their departmental affiliation, major advisor, thesis problem and an Abstract of their research is provided below:

a. Jeffrey M. Jones, Department of Molecular Virology, Lawrence A. Donehower, Ph.D., "Role of p53 in mammary tumorigenesis".

ABSTRACT

The progression of a normal cell or group of cells to what is defined as a tumor includes a wide variety of biological and genetic changes. Current tumor progression models describe this as a multistep process whereby cells undergo a series of changes before becoming fully malignant. Among the most common genetic changes in all types of cancer is the loss or inactivation of the tumor suppressor gene, p53.

In order to specifically address the role of loss of p53 in breast cancer and how this affects tumor progression we have developed a mammary tumor specific mouse model. We have crossed our p53 -deficient mice which are highly susceptible to a wide variety of early onset spontaneous tumors to Wnt-1 transgenic mice. The Wnt-1 transgenic mice carry a MMTV LTR driving mammary specific expression of a Wnt-1 transgene. The ectopic expression of this growth factor results in the development of mammary tumors in these mice. The Wnt-1 transgenic mice are a well established mammary tumorigenesis model. By crossing these two strains of mice we have established a new model in which we can specifically address the role of p53 in mammary tumorigenesis.

Using this model we have established that tumors which are deficient for p53 arise earlier and grow more rapidly than tumors with one or two wildtype alleles. The question we then wanted to ask is which function of p53 is responsible for this growth inhibitory

function. Using immunohistochemical techniques (TUNEL) we have shown that there is no difference in the levels of apoptosis observed in these tumors based on the presence or absence of p53. We have observed a significantly larger fraction of cells proliferating in p53-deficient tumors than in p53-positive tumors using both flow cytometry and mitotic figure counts. This suggests that p53s mechanism of inhibiting tumor growth rate in this model system is through its capacity to induce a cell cycle arrest and not through its ability to induce apoptosis.

Based on the observation that p53s primary mechanism of inhibiting tumor growth rate in this system is through its capacity to inhibit cell cycle progression we have crossed a second set of mice to test this observation in another biological system. We have crossed the Wnt-1 transgenic mice to the p21-deficient mice. p21 is a cyclin dependent kinase inhibitor which is activated by p53 in order to cause a G1 cell cycle arrest. By breeding Wnt-1 transgenic/p21-deficient animals we hope to show that the lack of p21 is enough to accelerate tumor growth rate even in the presence of wildtype p53.

b. Robin Weiss, Department of Molecular Virology, Ronald T. Javier, Ph.D., "Role of adenovirus type 9E4 ORF1 in mammary oncogenesis".

ABSTRACT

Unlike other adenoviruses, subgroup D Human adenovirus type 9 (Ad9) elicits exclusively estrogen-dependent mammary tumors in rats. An essential oncogenic determinant of this virus is E4 region open reading frame one (9ORS1), a gene encoding a 125 amino-acid protein and possessing cellular growth transforming activity. The objectives of my thesis work were to perform structure-function analyses on the novel 9ORF1 oncoprotein with the goal of revealing molecular mechanisms relevant to mammary oncogenesis.

Using CREF cells, an established rat embryo fibroblast cell line, I first generated cell lines that stably expressed the 9ORF1 protein (1). These 9ORF1-expressing cells were shown to differ from control CREF cells by displaying focus formation, morphological alterations, anchorage-independent growth, elevated saturation densities, and enhanced oncogenecity following injection into immunocompetent syngeneic rats. In addition, the results of immunofluorescence analyses with these cell line demonstrated that the 9ORF1 protein was located predominantly within the cytoplasm of cells. I also found that related E4 ORF1 proteins from subgroup A, B, C, and D adenoviruses possessed similar growth transforming potential, indicating that the human adenovirus E4 ORF1 polypeptides comprise a novel family of viral transforming proteins (2). Sequence database searches further revealed that the adenovirus E4 ORF1 proteins share weak sequence similarity with a variety of organismal and viral dUTP pyrophosphatase (dUTPase) enzymes. Despite lacking conserved dUTPase sequence motifs or detectable enzymatic activity, however, the adenovirus E4 ORF1 proteins were predicted to possess strikingly similar secondary structures with dUTPase proteins. Moreover, an avian adenovirus protein, encoded within a genomic location analogous to that of the human adenovirus E4 ORF1s was found to be a genuine dUTPase enzyme. These results hinted to the interesting possibility that the human adenovirus E4 ORF1 transforming proteins evolved from an ancestral adenoviral dUTPase gene.

To reveal 9ORF1 protein regions essential for cellular growth transformation, I next mutagenized the 9ORF1 gene (3). From a panel of 48 different mutant genes, seven 9ORF1 mutant proteins were identified that, in CREF cells, were impaired for inducing focus formation and growth in soft agar, as well as for enhancing tumorigenic growth in syngeneic rats. The sequence alterations of the seven mutant 9ORF1 polypeptides defined three separate 9ORF1 protein regions required for transformation: region I (residues 34-41), region II (residues 89-91), and C-terminal region III (residues 122-125). Because DNA tumor virus oncoproteins invariably function by binding to cellular growth-regulatory polypeptides, possible interactions between the 9ORF1 transforming protein and cellular factors were investigated (4). With *in vitro* experiments, several cellular phosphoproteins were detected that associated directly with wild-type 9ORF1 protein but failed to complex with the transformation-defective 9ORF1 C-terminal (region III) mutants. Ad5 and Ad12 E4 ORF1 transforming proteins also complexed with some of the 9ORF1-associated cellular polypeptides *in vitro,* as did fusion proteins containing 9ORF1 C-terminal protein fragments. More important, co-immunoprecipitation analyses demonstrated that the same cellular polypeptides associated with wild-type but not C-terminal mutant 9ORF1 proteins *in vivo*. These findings suggested that 9ORF1 C-terminal (region III) sequences, which are required for transformation, mediated direct binding of 9ORF1 protein to cellular polypeptides.

Sequence analyses subsequently revealed that 9ORF1 C-terminal region III contained a consensus PDZ domain-binding sequence motif (5). PDZ domains are conserved protein binding modules found in a variety of cellular proteins, some of which have been implicated in cell growth control and tumorigenesis. These observations led to the identification of DLG, a PDZ domain-containing polypeptide, as one of the 9ORF1-associated cellular proteins. Significantly, mammalian DLG is a functional homolog of the *Drosophila* discs large tumor suppressor protein, DLG-A. In summary, these studies indicated that the transforming activity of 9ORF1 may be mediated through its interaction with DLG, as well as with several other PDZ domain-containing cellular proteins. The critical role of 9ORF1 in the generation of mammary tumors by Ad9 further suggested that PDZ domain-containing cellular factors may play important roles in mammary cell growth control and, perhaps, in the development of breast cancer and other human malignancies.

c. Michael Mixon, Department of Cell Biology, Daniel Medina, Ph.D., "The role of *Brca1* in mouse mammary cancer".

ABSTRACT

A human gene linked to hereditary breast and ovarian cancer, *BRCA1*, was localized to chromosome 17q21 in 1990 and cloned through positional cloning methods in late 1994. The mouse homolog *Brca1* was cloned the following year. While initially observed to contain only two known protein motifs, an N-terminal C3HC4 zinc finger common to some differentiation-associated proteins and a bipartite putative nuclear localization signal, it has since been shown to contain a motif associated with DNA damage repair. All of these motifs are conserved between mouse and human. A proposed granin consensus sequence in the human protein is not conserved between mouse and human.

Expression of *Brca1* mRNA in the mouse mammary gland has been studied in virgin, pregnant and involuted samples and demonstrates a rise in expression during pregnancy as seen by others. Expression decreases after pregnancy to levels equal to or below those seen in the virgin animal, in contrast to the persistent elevation that has been reported. The expression pattern is similar in both Balb/c and FVB mice.

In order to experimentally manipulate *Brca1* expression, a full-length *Brca1* cDNA construct was required. Overlapping cDNA clones were used to construct a full-length *Brca1* cDNA, which is being used for expression studies *in vitro* and eventually *in vivo*. This construct generates a recombinant protein in reticulocyte lysates that runs at roughly 190kDa, near the predicted size of 198 kDa.

Four *BRCA1/Brca1* antibodies have been tested with the recombinant protein by immunoprecipitation and Western blotting. While all four were capable of precipitating recombinant protein, their effectiveness varied widely. More importantly, antibodies raised against amino acids 2-18 and 1793-1812 (extreme C-terminus) both recognized rBrca1, suggesting that the protein is full length. A preliminary Western blot showed that while at least some antibodies recognized labeled *rBrca1*, other unlabeled proteins were also detected indicating that there is some cross-reactivity.

Immortalized mammary epithelial cells have been stably transfected with Brca1 expression vector and are being characterized. If exogenous protein is being produced and can be confirmed as wild-type, these cells will be assayed for differences in growth rate and response to various genotoxic insults. Hyperplastic and tumorigenic cells are being transfected and will be analyzed similarly, with the addition of assaying effect on tumorigenicity. Antisense constructs to *Brca1* have been created and will be tested on similar cell lines to see if growth rates or degree of tumorigenicity are altered.

These constructs have likewise been placed under the control of a tetracycline inducible promoter for regulated expression. This may be necessary for the generation of stable transfectants in tumorigenic cells based on information from the human system, where overexpression of wild-type *BRCA1* has been said to prevent the generation of stable clones.

d. Shannon M. Lindsey, Department of Cell Biology, Jeffrey M. Rosen, Ph.D., "Role of STAT5 in mammary epithelial cell signaling".

ABSTRACT

STAT5 is a major protein involved in signaling of prolactin (Prl) and many other cytokines. There are two different STAT5 proteins, STAT5a and STAT5b, that are 96% identical but encoded by separate genes. Each isoform also has a splice variant, STAT5a2 and STAT5bd40C, which creates a carboxy-truncated form of the protein. I am interested in identifying functional differences between the full length STAT5 proteins, STAT5a1 and 5b, and the carboxy-truncated versions and in determining how these proteins act to regulate §-casein gene expression, mammary gland development, and if they have a role in tumorigenesis.

Because carboxy- truncated STAT proteins lack a transactivation domain but retain the portions of the protein required for dimerization and DNA binding, they are believed to act as dominant negative proteins. Studies on the effect of carboxy truncated STAT5 on the §-casein promoter as well as studies of other carboxy-truncated STATs, STAT1 and STAT3§, support this theory. STAT5a2 is unable to transactivate at the §-casein promoter in response to treatment with Prl alone. It also inhibits Prl-dependent induction of the §-casein promoter by STAT5a1 and STAT5b. STAT5a2 becomes phosphorylated in response to Prl treatment and does not affect the phosphorylation of STAT5a1 in response to Prl. STAT5a2 exhibits different DNA binding properties from STAT5a1 and STAT5b, however. A higher basal level of STAT5a2 binding to the §-casein GAS site is observed in the absence of Prl. Prolactin treatment leads to a marked increase in STAT5a2 binding to the GAS site. STAT5a2 also stays bound to the DNA following Prl treatment much longer than STAT5a1 or STAT5b. These data suggest that STAT5a2 acts in an inhibitory fashion by occupying the GAS site in place of the full-length proteins capable of transactivation. We hypothesized that STAT5a2 levels may change during the course of mammary gland development. Preliminary results from immunoprecipitation experiments using anti-STAT5 antibodies and mammary gland extracts suggest that the levels of STAT5a2 and STAT5bd40C remain relatively constant during mammary gland development. I would like to expand this analysis to investigate the levels of STAT5a2 and STAT5bd40C in mammary tumorigenesis. I am also constructing a transgene to overexpress STAT5a2 during late pregnancy and lactation by linking it to the whey acidic protein gene promoter.

Interactions with other transcription factors are also important for the actions of STAT5a1 and STAT5a2. We have found that glucocorticoid receptor (GR) enhances STAT5a1, STAT5b and STAT5a2 binding to the §-casein GAS site both on an isolated GAS site and in the context of a longer promoter fragment with several other transcription factor binding sites. Co-localization experiments in transiently transfected COS-1 cells have demonstrated that GR can be translocated into the nucleus in the absence of hydrocortisone by activated STAT5a1. Conversely, STAT5a1 can be translocated into the nucleus by hydrocortisone-bound GR in the absence of Prl. These data indicate a strong interaction between the two proteins. Our experiments compliment published studies which have demonstrated that STAT5a1 directly interacts with GR and GR acts synergistically with STAT5a1 to enhance transcription from the §-casein promoter. We are currently investigating whether the DNA binding is essential for GR/ STAT5 interactions by utilizing GR DNA binding domain mutants. We are also investigating the effects of GR on STAT5a2 inhibition of STAT5a1 activity. These studies on STAT5 and GR should reveal specifics about the interaction between the two proteins and will be important in understanding how GR can modulate the actions of STAT5.

e. William J. Jones, Department of Biochemistry, Stephen J. Elledge, Ph.D., Role of RAD53 in cell cycle checkpoints".

ABSTRACT

The ability to coordinate cell cycle transitions in response to genotoxic stress is critical to the maintenance of genomic stability. Mutations in mammalian genes that

brogate this response, such as p53 and ATM, cause a genetic predisposition to cancer. In yeast, several genes have been identified that control the cell cycle response to DNA damage, replication blocks or both, including the MEC, SAD, RAD, and DUN genes. DNA polymerase epsilon (POL2) is a potential sensor of DNA replication blocks that links the replication machinery to the S phase checkpoint. RAD53 encodes a protein kinase that controls cell cycle arrest and transcriptional responses to DNA damage and DNA replication blocks, including activation of the DUN1 kinase. MEC1 encodes a protein wit sequence similarity to lipid kinases that is involved in meiotic recombination and, like RAD53, the transcriptional and cell cycle responses to DNA damage and replication blocks. Mec1p belongs to the phosphatidylinositol kinase family that includes the S. pombe rad3 checkpoint gene, S. cerevisiae TEL1, required for telomere maintenance and the human ATM gene. ATM is mutated in patients with ataxia telangiectasia, a fatal disease characterized by autosomal recessive inheritance, immunological impairment, ataxia associated with progressive cerebellar Purkinje cell death and a high incidence of cancer. Approximately 1% of humans are heterozygotes and show an increased incidence of cancer. ATM defective cells show checkpoint defects similar to those of MEC1 and RAD53 mutants.

We have identified mutants of the S. cerevisiae ATM homolog MEC1 that can live only if the RAD53 checkpoint kinase was overproduced. I showed that MEC1 and TEL1 have overlapping functions in response to DNA damage and replication blocks that in mutants can be provided by overproduction of RAD53. Both MEC1 and TEL1 were found to control phosphorylation of RAD53p in response to DNA damage in collaboration with Dr. Yoli Sanchez. These results indicate that RAD53 is a signal transducer in the DNA damage and replication checkpoint pathways and functions downstream of two members of the ATM lipid kinase family. Since several members of this pathway are conserved among eukaryotes, it is likely that a RAD53-related kinase will function downstream of the human ATM gene and play an important role in the mammalian response to DNA damage.

f. Lilia Stepanova, Department of Biochemistry, J. Wade Harper, Ph.D., "Cdc37 as a rate-limiting step in mammary cell transformation".

ABSTRACT

Cell division is regulated through the activities of cyclin-dependent kinases (Cdks). There is now considerable evidence that deregulated cyclin expression and increased Cdk activity with inappropriate cell division is characteristic of cancer. As such, understanding the mechanisms underlying Cdk function is critical to our understanding of tumorigenesis. $p50^{Cdc37}$ has been recently identified as a regulator of Cdk4. This kinase is activated by D-type cyclins and overexpression of D-type cyclins in the breast induces tumors in transgenic animals. $P50^{Cdc37}$ is a subunit of Hsp90, a molecular chaperone and inactivation of Hsp90 function decreases the stability of newly synthesized Cdk4. Harper has proposed that the Cdc37/Hsp90 complex plays a central role in the establishment of pathways that are directly implicated in mammary transformation. Essentially all of the oncoprotein kinase components of the erbB2 pathway - including c-src homologs, Raf-1, and Cdk4 - have been shown to require Hsp90 function for stability and raf, src and Cdk4 are found associated with Cdc37/Hsp90 *in vivo*. In addition, there is indirect evidence that Hsp90/Cdc37 is required to stabilize erbB2.

Our central hypothesis is that Cdc37 is required to establish major protein kinase signaling pathways involved in mammary transformation and alterations in its expression in mammary epithelium constitutes a rate limiting event for mammary transformation by multiple oncogenic kinase pathways. Previous studies have shown that mammary transformation by ras, c-src, cyclin D1 and in some cases erbB2 in transgenic mice is stochastic, indicating that additional events are required for transformation in this setting. Our finding that Cdc37 is absent in resting mammary epithelium suggests that its induction may constitute one of the events required to support transformation by oncoprotein kinases. In keeping with our hypothesis, we have recently found that Cdc37 is absent in normal prostate epithelium but is induced in adjacent tumors and is readily detectable in some types of early disturbances in the prostate, including prostatic intraepithelial neoplasia, which may constitute pre-malignant lesions. This later finding, in combination with the cellular function of Cdc37, suggest that its induction may be an early step in the transformation process. To test this hypothesis, we have generated transgenic animals expressing Cdc37 in the breast using the MMTV promoter and in the prostate using the probasin promoter. We are currently at the stage of checking the animals for expression, analyzing any phenotypes associated with Cdc37 expression, and crossing animals with ras and cyclin D1 mice.

In addition, we have found that Cdc37 is a phosphoprotein and can be phosphorylated by casein kinase II on sites that are phosphorylated *in vivo*. We are currently making mutations in these sites to determine their functional significance.

This study will further our understanding of the mechanisms which establish signaling pathways and their role in transformation. The possible outcomes include the potential to use the level of Cdc37 expression in the breast in early diagnosis of the breast and other forms of cancer; and the elaboration of Cdc37 function could provide new clues to pharmacological treatment of cancer based on kinase activation.

B. Enhancement Programs

Two education programs specific for this training program have been functional for the past three years. The biweekly journal club in which students provide literature reviews and faculty/postdoctoral fellows provide research reviews for 1996 - 1997 is shown in Table II.

The course in "Molecular Carcinogenesis" is given every Winter bloc and each student has successfully passed (pass requires a grade of B or better) this course. The topic outline is shown in Table III.

C. Trainee Progress Review

Prospective trainees are nominated by letter and supporting documentation by a faculty member of the training program. The material on the prospective nominees are reviewed and selected by a standing subcommittee. Research progress of the trainees is reviewed by a standing departmental thesis committee, six faculty, which includes the major advisor and at

least one other member of the training grant. This committee meets twice yearly and submits a written report to the graduate school.

7. CONCLUSIONS

The training program in breast cancer is functioning as planned. Six students are currently enrolled and actively involved in their research phases of their training program. The breast cancer journal club meets every two weeks and it is anticipated that the involvement of additional laboratories will develop with the increased number of research projects on breast cancer due to new funding. In this past year, Dr. Wade Harper, Biochemistry has joined the participating faculty of our training Program. Dr. Harper is a recipient of a research grant from DOD. Additionally, three outside guest speakers, Dr. Dennis Slamon, UCLA, Dr. Clive Dickson, ICRF, England and Dr. Wen-Hwa, Lee, UTHSC, San Antonio, Texas, presented seminars and met with our students. This expansion should provide even greater exposure to our students to emerging problems in breast cancer.

8. PUBLICATIONS/MEETING ABSTRACTS

a1. Said, T.K., **Bonnette, S.**, and Medina, D. Immortal, non-tumorigenic mouse mammary outgrowths express high levels of cyclin B1 and activation of cyclin B1/cdc2 kinase. Cell Prolif. 29:623-639, 1996.

a2. Sanchez, Y., Desany, B.A., **Jones, W.J.**, Liu, Q., Wang, B., and Elledge, S.J. Regulation of RAD53 by the ATM-like kinases MEC1 and TEL1 in yeast cell cycle checkpoint pathways. Science 271:357-360, 1996.

a3. **Weiss, R.S.**, Gold, M.O., Vogel, H., and Javier, R.T. Mutant adenovirus type 9 E4 ORF1 genes define three protein regions required for transformation of CREF cells. J. Virol. 71:4385-4394, 1997.

a4. Lee, S.S., **Weiss, R.S.**, and Javier, R.T. Binding of human virus oncoproteins to hD1g/SAP97, a mammalian homolog of the Drosophila discs large tumor suppressor protein. Proc. Natl. Acad. Sci. USA 94:6670-6675, 1997.

a5. **Weiss, R.S.** and Javier, R.T. A carboxy-terminal region required by the adenovirus type 9 E4 ORF1 oncoprotein for transformation mediates direct binding to cellular polypeptides. J. Virol., (in press).

a6. **Jones, J. M.**, Attardi, L., Godley, L. A., Laucirica, R., Medina, D., Jacks, T., Varmus, H. E., Donehower, L. A. Absence of p53 in a mouse mammary tumor model promotes tumor cell proliferation without affecting apoptosis. Cell Growth & Differentiation., (in press).

a7. **Gangolli, E.A.**, Conneely, O.M. and O'Malley, B.W. Neurotransmitters activate the human estrogen receptor in a neuroblastoma cell line. J. Ster. Biochem. And Cell Biol., 1997, (in press).

b1. **Roy, D.**, Contreras, A., Coleman-Krnacik, S., and Rosen, J.M. Isolation of molecular markers for TEBS in mammary gland development. 9th International Conference on Carcinogenesis and Risk Assessment. Etiology of Breast and Gynecological Cancers, Barton Creek Resort, Austin, Texas, November 29 - December 2, 1995.

b2. **Chua, S.S.**, Wang, Y., DeMayo, F.J., and Tsai, S.Y. A bitransgenic mice model for breast cancer. Cancer Genetics and Tumor Suppressor Genes, Cold Spring Harbor, NY, August 14-18, 1996.

b3. **Gangolli, E.A.**, Conneely, O.M. and O'Malley, B.W. Neurotransmitters synergize to activate the human estrogen receptor in a neuroblastoma cell line. Tenth International Congress in Endocrinology, San Francisco, CA, P1:61, 1996.

b4. **Bonnette, S.G.**, and Medina, D. Characterization of the TGF-b1 response in a mouse mammary epithelial cell line and its transformed counterpart. Gordon Conference on Mammary Gland Biology, Plymouth State College, Plymouth, NH, June 15-20, 1997.

Table I. Status of Trainees 1994 - 1996

Student	Department	Advisor	Thesis Project	Status
Esha A. Gangolli	Cell Biology	B. W. O'Malley	Generation of a progesterone receptor transgenic mouse model	Graduated Fall, 1996. Current post-doctoral fellow; Univ. of Washington, Seattle.
Sharon G. Bonnette	Cell Biology	D. Medina	Mechanism of TGFβ1 inhibition of mammary cell growth	Scheduled to graduate December 1997.
Jeffrey M. Jones	Molecular Virology	L. Donehower	Role of p53 in mammary tumorigenesis	Scheduled to graduate Summer, 1998.
Annette C. Hollman	Molecular Virology	J. Butel	Oncogene co-operativity in mammary tumorigenesis	5th year student
Steven Chua	Cell Biology	M.-J. Tsai	Development of a bitransgenic mouse system to study oncogene function	4th year student
Deana L. Roy	Cell Biology	J. Rosen	Molecular markers for terminal end buds in mammary gland development	4th year student

Table II.

BREAST CANCER JOURNAL CLUB SCHEDULE
1996 - 1997

SEMINAR DATE	TIME	Room#	SPEAKER	DEPARTMENT	SEMINAR TITLE
09/25/96	12:00 - 1:00 pm	M616	Larry Donehower	Molecular Virology	p53 in mammary tumorigenesis
10/09/96	12:00 - 1:00 pm	M616	Susanne Krnacik	Cell Biology	FGFs in mammary development
10/16/96	12:00 - 1:00 pm	M616	Lilia Stepanova	Biochemistry	Journal report
11/13/96	12:00 - 1:00 pm	M616	Orla Conneely	Cell Biology	Lactoferrin
11/27/96	12:00 - 1:00 pm	M616	Shannon Lindsey	Cell Biology	Journal report
12/11/96	**4:00 - 5:00 pm**	**M112**	Dennis Slamon, Guest Lecturer	UCLA School of Medicine, Hematology/Oncology	to be announced
01/08/97	12:00 - 1:00 pm	M616	John Lydon	Cell Biology	Prg knock out mouse
01/22/97	12:00 - 1:00 pm	M616	William Jones	Biochemistry	Journal report
02/12/97	12:00 - 1:00 pm	M616	Raghu Sinha	Cell Biology	Mechanisms of selenium chemoprevention
02/26/97	12:00 - 1:00 pm	M616	Darryl Hadsell	CNRC	IGF-I and mammary development
03/12/97	12:00 - 1:00 pm	M616	Cindy Zahnow	Cell Biology	C/EBP and mammary tumorigenesis
03/26/97	**4:00 - 5:00 pm**	**M112**	Clive Dickson	ICRF, England	Cyclin D1 and mammary development/tumorigenesis
04/09/97	12:00 - 1:00 pm	M616	Mike Mixon	Cell Biology	Journal report
04/23/97	**4:00 - 5:00 pm**	**M112**	Wen-Hwa Lee, Guest Lecturer	Univ. of Texas Health Science Ctr., San Antonio	to be announced
05/14/97	12:00 - 1:00 pm	M616	Barry Markaverich	Cell Biology	Phytoestrogens
05/28/97	12:00 - 1:00 pm	M616	Robin Weiss	Molecular Virology	Journal report

TABLE III.

INTRODUCTION TO MOLECULAR CARCINOGENESIS
(D. Medina, J. Butel, L. Yeoman)

The course explores the fundamental concepts and experiments in tumor biology, cancer virology and oncogenes and growth control. This course provides a broad based introduction to students who have an interest in modern cancer research. Faculty from four departments (Cell Biology, Molecular Virology, Pharmacology and Biochemistry) serve as instructors. The course is open to graduate student and clinical fellows with a minimum requirement of Biochemistry or Cell Biology. Minimum number of students is 6. Lectures will be given 2X (Tuesday/Thursday) per week, 2 hours per session (1:00 - 3:00 pm) in room 187A.

LECTURE	DATE	TOPIC	LECTURER
1	1/7/97	Introduction/Pathogenesis/Epidemiology	DM
2	1/9/97	Cell Cycle and Cancer	WH
3	1/14/97	Growth Factors I: Stimulatory (EGF/TGFα/IGF)	LY
4	1/16/97	Growth Factors II: Inhibitory (TGFβ)	LY
5	1/21/97	Retroviruses	LD
6	1/23/97	Oncogenes I: Tyrosine kinases; receptors	LD
7	1/28/97	Oncogenes II: Nuclear factors	LD
8	1/30/97	Oncogenes III: Cancer and signalling	LD
9	2/4/97	Tumor Suppressor Genes I	BS
10	2/6/97	Tumor Suppressor Genes II	BS
11	2/11/97	Hepadnaviruses/Herpesviruses	BS/PL
12	2/13/97	Papovaviruses/Adenoviruses	JB
13	2/18/97	Chemical Carcinogenesis	DM
14	2/20/97	Tumor Progression and Metastasis	DM
15	2/25/97	Reversion and Inhibition of Carcinogenesis	DM
16	2/27/97	DNA Repair in Cancer	LD/
	03/04/97	FINAL	

Lecturers

Daniel Medina (DM) - Cell Biology
Wade Harper (WH) - Biochemistry
Janet Butel (JB) - Molec. Virology
Betty Slagle (BS) - Molec. Virology
Lynn Yeoman (LY) - Pharmacology
Larry Donehower (LD) - Molec. Virology
Paul Ling (PL) - Molec. Virology